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THE PLANT DISEASE SURVEY, DIVISION OF MYCOLOGY AND DISEASE SURVEY
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL ENGINEERING
AGRICULTURAL RESEARCH ADMINISTRATION
UNITED STATES DEPARTMENT OF AGRICULTURE

SUPPLEMENT 141

COTTON SEEDLING DISEASES AND BOLL ROTS
DISTRIBUTION AND DISSEMINATION

April 1, 1943

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Division of Mycology and Disease Survey serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

COTTON SEEDLING DISEASES AND BOLL ROTS
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Plant Disease Reporter
Supplement 141 April 1, 1943

The following reports, by Paul R. Miller of the Division of Mycology and Disease Survey, and by Richard Weindling of the Division of Cotton and Other Fiber Crops and Diseases, summarize the results of 4 years of surveys for cotton diseases and of special studies on distribution and dissemination suggested by observations made during the surveys.

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A SUMMARY OF FOUR YEARS OF COTTON SEEDLING AND BOLL ROT DISEASE SURVEYS

Paul R. Miller

Prior to the initiation of these cotton disease surveys, seed treatment was known to be effective in controlling certain cotton seedling diseases; however, explanations for the results obtained were largely empirical since the organisms involved in the seedling-disease complex were definitely known only for a limited area. It appeared, therefore, that studies of a regional scope, over a period of several years, concerning the identity, distribution, and importance of the fungi associated with damping-off of cotton seedlings and with boll rots, should give a more effective basis for research on specific seed treatments for the different cotton-producing areas.[1]

Hence these surveys, in which samples were obtained in 14 States and from more than 2000 fields, were begun. They involved the cooperation of both Federal and State pathologists, to whom the author expresses his sincere appreciation.

Because of the present emergency it has been necessary to discontinue these surveys, therefore a brief summary of the results so far obtained, during the growing seasons of 1938 to 1941 inclusive, is presented herein. Detailed reports have been issued upon completion of each separate survey (1, 2, 3, 4, 5, 6, 7, 8). Methods employed, as well as a discussion of the first 2 years' findings, have also been presented (10). Portions of the following discussion have already appeared in the reports cited and in preparing this summary no especial attempt was made to avoid repetition.

Perhaps the most interesting finding made during these surveys concerns the wide distribution of Glomerella gossypii Edg. throughout the Southeastern States on both diseased seedlings and bolls. It occurred on 81.2% of the seedling samples and on 67.8% of the boll samples (Tables 1 and 2). However, in Texas and Oklahoma occurrence of this fungus was limited to the eastern portion of each State. Maps showing this distribution have been shown in various issues of the Plant Disease Reporter.

Failure to find the anthracnose fungus in the Western Belt is evidently attributable to unfavorable dry conditions which prevent its survival during the period between the damping-off stage and the boll-rot stage (Figure 1). Previous to our surveys it was assumed generally that anthracnose practically disappeared with the advent of the boll weevil, which had forced the adoption of early-maturing cotton varieties of open type. Perhaps failure to recognize that the epidemiology of boll rots has changed considerably since the pioneer work on anthracnose accounts for this erroneous assumption. In our collections symptoms on the majority of

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1 This information now has an especial timeliness, also, in view of the anticipated shortage of certain chemicals used in the manufacture of seed treatment compounds.

Table 1. Fungi isolated from cotton seedling samples collected in 1938, 1939, 1940, and 1941

State	Number of samples examined	Number of samples found with: Glomerella gossypii	Fusarium moniliforme	Rhizoctonia solani	Fusarium spp.	Diplodia gossypina	Other fungi [a]
Alabama	151	137	138	15	60	4	57
Arkansas	129	119	121	34	49	3	66
Florida	20	19	16	3	20	4	24
Georgia	139	132	129	17	47	7	72
Kentucky	5	4	4	0	1	0	5
Louisiana	133	114	102	30	70	2	82
Mississippi	183	151	134	43	108	6	116
Missouri	5	5	5	0	2	0	1
North Carolina	67	49	43	4	35	3	54
Oklahoma	61	11	46	7	29	3	71
South Carolina	275	219	215	38	127	7	117
Tennessee	56	51	38	6	40	3	16
Texas	69	37	54	10	29	1	63
Virginia	59	50	50	3	31	2	33
Total	1352	1098	1095	210	648	45	777
Percentage		81.2	81	15.5	47.9	3.3	57.4

[a] Includes Alternaria spp.; Penicillium spp.; Aspergillus spp.; Pythium spp.; Sclerotium bataticola; and Sclerotium rolfsii.

Table 2. Fungi isolated from cotton boll samples collected in 1938, 1939, 1940 and 1941

State	Number of samples examined	Number of samples found with: Glomerella gossypii	Fusarium moniliforme	Alternaria spp.	Fusarium spp.	Diplodia gossypina	Other fungi [a]
Alabama	69	50	38	57	32	10	46
Arkansas	81	53	48	77	47	8	31
Georgia	83	71	62	77	48	29	60
Kentucky	4	4	1	4	4	0	0
Louisiana	44	34	25	24	31	11	30
Mississippi	223	193	180	168	193	35	117
Missouri	8	2	5	8	5	0	2
New Mexico	2	0	0	2	0	0	1
North Carolina	66	53	40	62	19	4	39
Oklahoma	77	5	35	76	41	4	31
South Carolina	211	169	147	198	113	26	127
Tennessee	19	7	6	19	12	1	10
Texas	98	19	50	93	58	1	27
Virginia	44	38	27	35	18	6	29
Total	1029	698	664	900	621	135	550
Percentage		67.8	64.5	87.5	60.3	13.1	53.4

[a] Aspergillus spp., Rhizopus spp., and Penicillium spp.

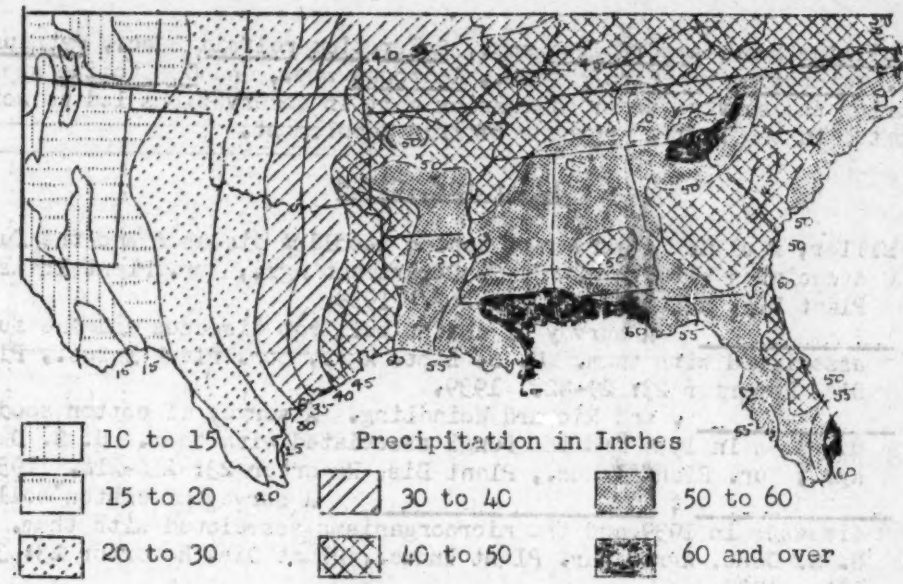

Fig. 1. Annual rainfall in the cotton belt of the United States

specimens from which the anthracnose fungus was cultured did not conform to those described earlier, in that the lesions were usually much smaller, they were not sunken and brownish in color with a red border, and only rarely were the moist, pinkish, pasty looking masses of spores encountered. Most boll lesions from which G. gossypii was isolated were limited spots, usually water-soaked in appearance, and indistinguishable from those caused by the angular leafspot bacteria (Xanthomonas malvacearum), or by other microorganisms. Field observations and other evidence obtained during these surveys indicated that X. malvacearum plays an important role with respect to the anthracnose boll disease by providing infection courts for G. gossypii (9). Apparently boll weevil punctures are also courts of entry, particularly during the latter part of the season.

Fusarium moniliforme Sheld. and other species of Fusarium occurred on both seedlings and bolls about as frequently as did G. gossypii. However, it is difficult to evaluate the significance of the high percentage of F. moniliforme since this fungus does not appear to be a primary pathogen of damping-off of cotton seedlings. This is true also for the various other species of Fusarium isolated, since little is known regarding their pathogenicity to cotton seedlings. On bolls these Fusaria, as well as Alternaria spp., are considered mainly as secondary invaders, entering the bolls through lesions caused by other agents.

Rhizoctonia solani Kühn, which is a virulent seedling pathogen, although isolated from a relatively small number of samples (15.5%), was found to be rather widely distributed.

The occurrence of the following fungi, whose pathogenic effects are not so well known, was sufficiently common to deserve mention: Diplodia spp.,

Penicillium spp., Aspergillus spp., Sclerotium rolfsii Sacc., Pythium spp., Sclerotium bataticola Taub., Rhizopus spp., and Chaetomium spp.

During the surveys under discussion certain observations led to work reported in the next 4 articles of this Supplement.

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(DIVISION OF MYCOLOGY AND DISEASE SURVEY. CHAIRMAN, COTTON DISEASE SURVEY COMMITTEE).

OCCURRENCE OF THE ANTHRACNOSE FUNGUS, GLOMERELLA GOSSYPII, ON COTTON PLANTS GROWN FROM INFESTED SEED AT FOUR LOCATIONS IN 1941

Richard Weindling

This report deals with the epidemiology of the anthracnose disease of cotton between seedling and boll phase. Two pertinent problems have been brought into bold relief by the seedling and boll disease surveys: 1) How does the anthracnose fungus survive and spread during the summer months? and 2) Is its scarcity in western Texas and Oklahoma due to the prevailing summer climate?

With respect to the second question, the available evidence is mostly circumstantial. Present conditions have brought about abandonment of plans along those lines of the work which were expected to furnish adequate data.

The first question has been answered to some extent by Atkinson (2) as early as 1892. He found the fungus fruiting on dead portions of leaves and stems, particularly around leaf scars. Later investigators confirmed this observation. More recently, culture work has supplemented these data. In 1938, a high percentage of plants used in experiments on Fusarium wilt at Clemson, South Carolina, yielded cultures of the anthracnose fungus. It was obtained from basal portions of surface-disinfected stems. Subsequently, the fungus was cultured from apparently healthy tissues of seedlings that had been inoculated with G. gossypii (4), and from stems, petioles, and leaf glands of mature field-grown plants (1). Such "latent" infection is known in anthracnoses of some other plants. In such infected tissues, the fungus is ready for sporulation whenever some of the tissue breaks down and moisture conditions are favorable.

The procedure chosen to investigate the aforementioned problems was the following: 1) To gain a well-rounded picture of occurrence and spread of the anthracnose fungus, portions of cotton plants grown from infested seed were cultured and examined at frequent intervals during the growing season; 2) To study the effect of climatic conditions, comparable material was secured from several locations.

Materials and Methods

Seed of the variety Miller, heavily infested with the anthracnose fungus, was planted at the following Experiment Stations: Florence, South Carolina (coastal plain); Clemson, South Carolina (Piedmont); Knoxville, Tennessee; and Temple, Texas (black land)[1].

Whole plants or portions of plants chosen at random were collected at intervals during the season. Samples from Florence and Clemson were brought directly to the laboratory. Except for the last shipment, samples from Texas were forwarded by air mail.

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[1] The writer gratefully acknowledges the cooperation of Paul R. Miller, Knoxville, and C. H. Rogers, Temple, who planted the seed and collected the material from these two locations.

On arrival in the laboratory the specimens were treated as follows: Pieces were cut from seedlings, stems, and bolls. They were surface-disinfected, cultured, and examined as described elsewhere (4). The material taken from the stems of plants after the thinning stage consisted of uninjured bark portions including a part of a leaf scar when possible. These sections were cut at 2 or 3 levels of the stem, the lowest at the soil surface or at the cotyledonary node, and the highest at the second woody node. Leaves were wrapped separately in moist paper towels and incubated for 2 days in moist chambers at 24°C. Washings from the leaves were mounted on glass slides and observed for fungous spores. Leaf petioles, corollas, and bracts were similarly treated, the latter without previous incubation.

Data were taken on 25 or 50 specimens, when available. With respect to numbers of specimens as well as of samples, those from Clemson and Temple were inadequate. Stands of these 2 plantings were poor, and development of the plants was not uniform. At Clemson dry weather delayed germination, while at Temple, moisture conditions made replanting necessary. Excepting the first sample, material from Temple came from both original and replant plantings.

Results

A summary of results is presented in Table 3 in which occurrence of Glomerella gossypii is listed as percentages of specimens examined. The most obvious feature of this summary is the frequency of positive observations in the samples from Knoxville and Florence as contrasted to those from Clemson and Temple. It appears that, even when adequate samples were secured from the latter 2 locations and the anthracnose fungus was found, it was not present in a high percentage of specimens, except at the seedling stage. The most interesting single result is the appearance of the fungus in the last sample from Texas.

From the data presented here, it is difficult to draw conclusions regarding the relative frequency of the fungus on the various plant organs. Previous work had demonstrated that development and detection of spores on leaves is greatly facilitated by incubating dead or injured portions in moist chambers. When the bracts were placed in a moist chamber, the percentage of G. gossypii was raised above that for leaves of the same sample. In general, cultures from the middle portion of the stem yielded the fungus more frequently than those from the basal part. Competing soil organisms may play a role with respect to isolation and survival in the basal part. Isolations from petioles were secured, but not regularly. In one case (last Temple sample), the percentage of Glomerella-infected petioles was higher than that of the leaves. Spores of the fungus have been found frequently in the dead tissues of nectar glands of leaves and petioles. Perhaps these glands deserve the special emphasis placed upon them by some observers of summer survival (1).

Summaries of weather data from the 4 locations are presented in Tables 4 and 5 for consideration in conjunction with the results summarized in Table 3. It is apparent at first glance that simple and general correlations can not be derived from these 3 tables.

Table 3. Percentage occurrence of Glomerella gossypii on cotton plants grown from infested seed (var. Miller) in 4 locations in 1941

Location and Date of collection	Tissue cultures from portions of stem			Leaves	Corollas	Bracts	Bolls	
	Upper	Middle	Lower				Culturing	Scraping
Clemson, S.C. (planted May 3)								
June 2	-	-	60.0	-	-	-	-	-
June 26	0	0	4.0[a]	40.0[b]	-	-	-	-
July 24	0	0[a]	0	28.6	-	-	-	-
August 7	-	-	-	50.0[b]	20.0	-	-	-
August 21	0	0[a]	0	8.6	0	0	0	-
September 5	-	-	-	-	-	30.0[b]	24.0	-
October 10	8.0	0[a]	0[a]	18.0[a]	-	8.0	-	8.0
Florence, S.C. (planted April 15)								
April 29	-	-	26.7[b]	-	-	-	-	-
May 15	-	-	25.6	-	-	-	-	-
June 12	0	0	16.0	-	-	-	-	-
July 2	4.0	2.0	0.0[a]	10.0[b]	0[b]	-	-	-
July 19	16.0	16.0	-	85.0	52.0	-	15.0	-
August 13	52.0	80.0[a]	20.0	85.0[a]	-	60.0	60.0	-
August 28	88.0	96.0	28.0	95.0	-	100.0	86.6	-
Knoxville, Tenn. (planted April 30)								
May 25	-	-	72.0	-	-	-	-	-
June 20	-	-	36.0	-	-	-	-	-
July 7	8.0	40.0	18.0[a]	60.0[b]	-	-	-	-
July 26	-	8.0	18.0	32.0	0[b]	-	0[b]	-
August 11	-	12.0	24.0	56.0	-	20.0	10.0	-
August 30	-	64.0	32.0	100.0	-	100.0	40.0	-
September 14	-	-	-	60.0	-	30.0	25.0	-
October 9	32.0	34.0[a]	12.0[a]	68.0[a]	-	29.5[a]	-	10.0[a]
Temple, Texas (planted about April 25 and May 25)								
May 12	-	-	45.7	-	-	-	-	-
July 5	0	0[a]	0	-	-	-	-	-
July 28	-	0[b]	-	0	0	-	-	-
September 9 & 15	0[b]	-	-	0[b]	-	0[b]	0[b]	-
September 25	0	8	2[a]	24.0[a]	-	5.0[a]	-	8.0[a]

Note: Percentages based on observations of 25 specimens, except as indicated in footnotes:

[a] Percentages based on 50 observations.

[b] Percentages based on 10-15 observations.

Table 4. Monthly rainfall and days with rainfall at the 4 locations

Month	Clemson		Florence		Knoxville		Temple	
	Rainfall in inches	Days with .1 in. or more	Rainfall in inches	Days with .1 in. or more	Rainfall in inches	Days with .1 in. or more	Rainfall in inches	Days with .1 in. or more
		3		1		4		3
		4		5		5		7
April	1.61	9	3.02	6	3.95	21	6.21	19
		23		11		24		21-28
		24		25		25		
		7		8		8		3
						31		5
May	.72		.67		0.52		4.15	11
								19
								21
		2		3				3
		3		4		10-15		6
		7		11-13				7
June	2.69	10	8.75	15	2.49		6.25	10
		11		21				16
		22		23-29				26
		23						27
		3-7		5-7		3-7		4
		14-16		11		9		11
July	6.56	19	6.33	13	7.24	10	3.80	12
				15-19		15		14
				23		17		
						20		
		1		4		5		
		3-5		17		13		4
August	5.90	19-21	5.83	21	3.14	16	2.70	6
				23		26-28		7
		27		24				
				5				16
		24-26		6		5		23
Sept.	.63		1.62	26	0.42	10	0.44	
				27		13		
Total	18.11		26.22		17.76		23.55	

Table 5. Monthly average mean temperatures at the 4 locations

Month	Mean temperature (°F.)			
	Clemson	Florence	Knoxville	Temple
April	63.11	65.2	62.8	66.8
May	71.5	71.7	68.9	74.8
June	76.7	77.2	74.8	79.1
July	79.0	80.5	79.4	82.5
August	79.7	79.3	79.2	83.6
September	75.1	77.0	75.8	80.9

The trends of the Florence data are rather easily explained. Rapid emergence kept seedling infection low. In July, the fungus began to spread rapidly. Owing to frequent rains, corollas adhered to the tips of bolls for weeks forming an incubation chamber for Glomerella (found on 52% of the corollas of one sample) and other fungi. Many early bolls rotted from the tip down, a condition causing severe damage in the Coastal Plain Section. Heavy aphid infestation in August covered all aerial plant organs with honey-dew which provided an ideal medium for the growth of the anthracnose fungus. This, together with progress of the weevil on bolls, may account for the progressive increase in frequency up to the last sampling.

A similar "building-up" trend is noticeable in the Knoxville data from late July through August. The fungus was found, however, from the seedling stage until July on a fairly high frequency level. The decrease in the fall may be connected with lack of rainfall, absence of boll weevil, and with a leaf worm infestation which practically defoliated the plants.

At Clemson, climatic conditions throughout the season were very similar to those at Knoxville. Nevertheless, frequency percentages of the fungus were generally lower. A possible explanation of this divergence is that the irregular growth and stand of the plants did not provide sufficient shade to prevent the plant parts from drying very rapidly.

In the first Temple planting, anthracnose injury to seedlings was high. Summer survival of the fungus is considered uncommon in this section of Texas. The somewhat unusual development on seedlings was attributed to an abnormally rainy spring. The data from the limited number of samples submitted give some indication that over-summering was more difficult than in the eastern locations. This is probably attributable to the small number of rainy days in July and August at Temple.

Discussion

Students of the anthracnose diseases of bean and cucumber have demonstrated that the causative fungi are able to remain dormant and thus survive for long periods as mycelium in tissues or as appressoria attached to the surface. With respect to the dissemination of these fungi, rain is considered the principal agency. The spores are spread by run-off water over the soil surface where they have been found to retain their viability for

several days. Rain drops splash spores from leaf to leaf, from plant to plant, and from the soil on to the plants. The fungi become most easily established in tissues affected by some kind of injury. Infection is favored by moderate temperature and high moisture, the same conditions that benefit subsequent sporulation.

This evidence, though obtained principally for other anthracnoses, is applicable as a general background for the data presented here. The cotton anthracnose fungus has survived in 1941 at all 4 locations. It remained well distributed and active throughout the season at Knoxville, fluctuated at a lower level of frequency at Clemson, remained quiescent during most of the season at Temple, and built up rapidly after an initial latent period at Florence. The fungus seemed to be capable of rapid spread whenever conditions became favorable, and, if they remained so over a considerable period, it built up to epidemic proportions (Florence). Periods of rainfall and rather cool, cloudy weather are no doubt primary factors for dissemination. Of equal importance, however, seem to be factors connected with the saprophytic habit of the fungus. Chief among these appears to be the availability of injured, dying, or dead plant tissues which usually become more abundant as the organs of the plant mature. Lesions caused by other disease organisms or insects may play a significant role. Furthermore, close stands and large plants tend to provide more shade than open stands and small plants, and therefore more decomposing material and a more favorable micro-climate for the fungus (Knoxville and Florence vs. Clemson and Temple).

It is noteworthy that pink spore masses, commonly considered as a sign of the anthracnose disease, were not observed on any of the stem or leaf portions which on culturing or incubation yielded the anthracnose fungus. Similarly, seasoned observers agreed that the severe rot of early bolls occurring in 1941 in eastern South Carolina was not pink boll rot or anthracnose. However, in fields of this section planted with Ceresan-treated seed, the anthracnose fungus was found on rotted bolls as well as on other plant organs nearly as often as in the collections from Florence analyzed in this study. Evidently, the fungus frequently occurs in association with other organisms that tend to obscure outward signs of its presence. The importance of these potential or hidden sources of inoculum will be emphasized further by portions of the following article dealing with the role of trash in seed infestation during ginning.

Summary

Evidence has been obtained on the presence and spread of the anthracnose fungus during the 1941 season, and on the influence of climatic factors, by examining and culturing, at intervals, portions of cotton plants grown from infested seed at 4 locations. The fungus was found at all locations in the seedling and boll stage, and at the 3 eastern locations throughout the summer on stems, leaves, and bracts. Simple relationships between climatic factors and relative abundance of the fungus could not be established. The data indicate that, in addition to periods of rainfall, other factors are important, such as the availability of dead plant tissues and of shade provided by close stands of large plants. It is suggested that

the fungus over-summered at Temple, Texas, in a quiescent form, and that unusually wet spring weather gave a better chance for its persistence than in ordinary years.

In the eastern parts of the cotton belt, the anthracnose fungus frequently infects stems, leaves, and other organs of the cotton plant. When moisture conditions become favorable, such latent infections are followed by saprophytic development in rotting tissues that provide potential sources of inoculum for boll infection and seed infestation.

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RELATION OF GINNING TO CONTAMINATION OF COTTON SEED BY THE ANTHRACNOSE FUNGUS

Richard Weindling and Paul R. Miller

During the cotton disease surveys, the observation was made that the number of bolls infected with the anthracnose fungus, Glomerella gossypii, was very small when considered in relation to the frequency of the fungus as a seedling parasite. Often, most of the seedlings in large fields had anthracnose lesions, but it was rare during any of the 4 yearly boll surveys to find a field with as many as 1% of the bolls infected. The assumption that the seed is the principal source of seedling inoculation is supported by the effective results of seed treatment. It seems improbable, however, that infected seedlings originate only from the seed derived from so few diseased bolls.

The theory was advanced that seed may become contaminated with the anthracnose fungus during ginning. Exploratory examination of materials collected from gins near Clemson, South Carolina, made it manifest that "trash"[1] carries even more conidia of G. gossypii than seed. In modern gins most of this trash is removed from fiber and seed by extracting and cleaning equipment. These operations are accompanied, however, by vigorous whirling and mixing of the seed cotton. It should be expected that spores and small particles carrying the fungus are effectively distributed during ginning, just as agitation spreads germicides in seed treatment operations. Seed free from G. gossypii may thus become contaminated by

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[1] The term trash is used here to designate broken bolls, hulls, pieces of leaves, stems, bracts, petioles, and other debris that has been picked with the seed cotton.

spores originating not only from other seed and from bolls but also from infected plant organs contained in the trash. Furthermore, particles of trash may adhere to machinery of the gin, attach themselves to seed of lots ginned subsequently, and thus contaminate them.

The data presented here are concerned principally with (1) findings of conidia of Glomerella on samples of seed and trash collected from gins in South Carolina during the past 3 seasons, and with (2) supplementary work on seed contamination produced by adding contaminated trash to seed cotton prior to ginning. The spore load determinations are quantitative, and reveal some effects of sectional, seasonal, and climatic factors on seed contamination. Such data may be useful as a background for further investigations along this line.

Materials and Methods

Samples of seed and trash were brought to the laboratory in paper bags and stored until used. Glomerella contamination was determined by 2 methods: (1) germinating 2 aliquots of 100 seed in flats with steamed sand in the greenhouse, and (2) counting spores washed from the seed or trash as described below. These two methods were also used with seed resulting from the experimental ginning.

In 1939, all samples were taken at one gin in the upper Piedmont, 2 samples of seed and trash being derived from a given lot of seed cotton. In 1940 and 1941, samples were collected from numerous gins throughout South Carolina, one sample of seed and trash per gin[2]. Conditions at most gins made it impracticable to secure seed and trash from the same lot of seed cotton.

The procedure for counting conidia of Glomerella gossypii was essentially as follows. One hundred grams of seed or 40 grams of trash were shaken vigorously with 300 cc. of water in quart jars. The washings were passed through cheese cloth. Washings from seed were centrifuged to 1/100 the original volume. Spore counts were made of these concentrates and of the trash washings with a haemocytometer commonly used in counting spores or blood cells. Determinations from 2 drops were usually found sufficient. If they varied considerably or if Glomerella spores were absent, results from additional mounts were averaged to obtain an estimate of spore load. This method has been employed from 1939 to 1941 by the senior writer with minor modifications. The junior writer has contributed to this study the 1941 data on spore load of seed. He has used an improved version of the above method as described by him in detail in a previous report, (P.D.R. 24: 85. 1940).

Results of Collections from Commercial Gins in 1939, 1940, and 1941

The principal features of the data assembled in tables 6-8 and of concurrent observations may be expressed as follows:

(1) All washings of trash samples (except one) yielded conidia of G. gossypii. Samples with a preponderance of fine trash (broken leaves,

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2 Thanks are due to Mr. C. C. Bennett for collecting many of these samples.

Table 6. Glomerella contamination of samples collected from a gin in the Upper Piedmont of South Carolina in 1939

Seed Cotton lot no.	Sample no.	Seedling disease index [a]	Spores of G. gossypii	
			Per seed	Per mg. trash
1	1	85	268	463
	2	58	296	314
2	1	81	19	420
	2	84	19	108
3	1	49	16	177
	2	55	28	36
4	1	59	0	223
	2	65	0	156
5	1	61	0	59
	2	58	6	183
6	1	81	0	52
	2	84	0	17
7	1	69	88	297
	2	70	32	389
8	1	52	32	296
	2	59	19	223
9	1	79	161	320
	2	82	153	228
10	1	73	120	297
	2	69	19	263

[a] Index obtained by subtracting from 100 the percentage of healthy and 1/2 the percentage of lesioned plants.

bracts, and other debris) had spore loads varying in the same range of magnitude as those of coarse trash (hulls, broken bolls, pieces of stems). This might have been expected from the findings of G. gossypii on leaves and other organs of field plants discussed in the preceding article.

(2) In all seed samples without exception, presence of the anthracnose fungus was demonstrated by microscopic examination of lesioned seedlings obtained in the germination test. Glomerella gossypii caused most of the pre- and post-emergence losses combined in the tables as a disease index.

(3) Spore loads of the anthracnose fungus on seed samples varied more widely than disease indices. Spores were not detected by the washing-centrifuging method in several samples that had considerable Glomerella damping-off when germinated.

(4) Spore loads on seed as well as on trash were generally higher in 1941 than in 1940. The spore loads in samples from the Coastal Plains section exceeded those from the Piedmont in 1941, but not in 1940. Evidence given in the preceding article suggests that rainfall in June, July, and August is a decisive factor with respect to spread of Glomerella in the field. In 1940, June and July rainfall in South Carolina was below normal.

Table 7. Glomerella contamination of samples collected from South Carolina gins in 1940. Nos, 1-14 are from the Piedmont, Nos. 15-24 from the Coastal Plain Section

Sample no.	Seedling Disease Index [a]	Spores of G. gossypii	
		Per seed	Per mg. trash
1	42.0	0	85
2	45.3	0	120
3	85.8	117	780
4	53.5	12	128
5	53.6	16	280
6	49.3	0	64
7	49.4	59	48
8	48.2	18	32
9	67.3	0	60
10	73.9	24	11
11	84.4	39	20
12	80.7	169	496
13	84.4	813	416
14	63.2	29	144
15	85.7	157	70
16	46.7	40	47
17	74.0	146	520
18	57.9	8	0
19	74.3	513	688
20	74.3	15	40
21	66.7	19	190
22	43.0	42	200
23	85.3	78	325
24	76.7	32	540

[a] See footnote Table 6

In 1941, it was considerably above normal, particularly in the Coastal Plain. August rains were about normal in 1941, and much above normal in 1940, but in the latter case they fell in a few days of heavy storms.

(5) The viability of many seed of the Coastal Plain samples of 1941 was low, as indicated by germination of delinted seed (Table 8). Internal infection was present in some seed of all these samples, but only in few samples from the Piedmont. Correlations were not noticeable among the data on viability, internal infection, disease index, and spore load. The absence of correlations may be due to the heterogeneity of the samples. Some duplicate samples from the same gin even showed considerable variation (Table 6).

Table 8. Glomerella contamination of samples collected from South Carolina gins in 1941. Nos. 1-22 are from the Coastal Plain, Nos. 23-43 from the Piedmont

Sample no.	Percent non-viable seed	Disease index [a]	Spores of G. gossypii	
			Per seed	Per mg. trash
1	40	72.3	80,000	3,575
2	49	74.5	4,000	3,028
3	43	89.5	1,000	6,133
4	26	80.0	32,000	4,916
5	38	77.8	48,000	3,268
6	40	76.0	64,000	7,110
7	52	85.0	2,000	11,523
8	49	74.8	0	1,875
9	37	85.8	0	6,660
10	35	81.3	16,000	1,738
11	18	54.5	0	4,745
12	16	54.5	2,000	5,430
13	36	80.0	48,000	6,543
14	35	83.8	16,000	3,413
15	10	82.8	4,000	6,483
16	18	78.0	4,000	3,145
17	17	64.8	16,000	2,833
18	31	86.5	32,000	4,590
19	23	76.3	2,000	1,348
20	51	94.3	trashy	3,425
21	24	84.0	80,000	4,443
22	14	73.3	160	2,785
23	9	56.0	3,000	980
24	4	94.0	1,000	508
25	6	97.0	60,000	548
26	7	53.5	160	13
27	7	85.3	3,000	3,035
28	5	68.3	1,000	2,690
29	5	82.3	3,000	2,175
30	9	67.8	0	155
31	11	60.3	0	100
32	8	80.8	120	118
33	2	81.5	4,000	93
34	6	47.5	200	52
35	14	62.0	3,000	483
36	11	49.0	2,000	483
37	16	48.0	160	885
38	8	65.5	0	938
39	9	34.0	0	13
40	8	22.5	80	313
41	78	84.8	0	430
42	6	51.5	48,000	1,095
43	6	64.3	16,000	1,600

[a] See footnote Table 6.

Table 9. Averages of data from 1940 collections

Section	Number of samples	Seedling disease index [a]	Spores of G. gossypii	
			Per seed	Per mg. trash
Piedmont	14	62.9	91	192
Coastal Plain	10	68.5	105	262
Piedmont and Coastal Plain	24	65.2	98	221

Table 10. Averages of data from 1941 collections

Section	Number of samples	Percent germination of delinted seed	Seedling disease index [a]	Spores of G. gossypii	
				Per seed	Per mg. trash
Piedmont	21	88.8	64.6	6,891	796
Coastal Plain	22	68.1	77.7	21,484	4,500
Piedmont and Coastal Plain	43	78.2	71.3	13,858	2,691

Table 11. Effect of trash on seed contamination with G. gossypii. One-half pound of trash was added to each 5 lb. lot of seed cotton, except to control lots, prior to ginning. Lots ginned in order of numbers

Lot Number	Trash added		Disease index [a]	Spores of G. gossypii per seed
	Kind	Spore Load		
1	control	---	21.5	--- [b]
2	fine	low	22.0	--- [b]
3	fine	medium	39.5	23
4	fine	high	82.3	410 [b]
5	control	---	36.0	---
6	coarse	high	84.0	1,000 [b]
7	control	---	37.5	---
8	coarse	very high	80.5	88,000
9	control	---	88.3	138

[a] See footnote Table 6.
[b] Spores found; number below 12 per seed.

Results of Ginning Experiments

The findings on trash with regard to frequency and abundance of conidia of G. gossypii led to attempts at inducing seed contamination during ginning by the addition of trash. A preliminary test in 1939 gave positive results. An experiment was conducted in 1940 in order to gain information regarding (1) extent of contamination of trash in relation to that of seed, and (2) carry-over of Glomerella from contaminated to non-contaminated lots. The seed cotton was divided into aliquot lots. They were ginned in the order given in Table 11, after some of them had been amended with weighed amounts of trash. These trash samples were secured during the surveys and their degree of contamination was as indicated in Table 11. With increase in the spore load of the trash contamination also increased. Highly contaminated trash brought about very severe seed contamination as well as carry-over to the lot ginned subsequently. In the samples ginned after the lots that had become less heavily contaminated, some carry-over was evident in seedling infection but not in spore load. Without doubt, carry-over would have been larger in commercial gins where seed and trash are not removed from the gin after each sample as was done in the experiments.

The gin used in this experiment was a small hand gin that had only the essential parts of modern gins, gin saws and roll box or gin breast. A comparable experiment on a gin equipped with feeding and cleaning machinery of modern gins was made possible in 1941 at Knoxville, Tennessee. A lot of 50 lb. of seed cotton was ginned while adding 4 lb. of heavily infested trash. The resulting seed became contaminated with the anthracnose fungus at the rate of 2318 spores per seed (average of 22 determinations). Seed from the original seed cotton was slightly contaminated but the spore-load determination was negative.

Discussion

Seed contamination with Glomerella has been indicated here by 2 data, disease index and spore load. It is realized that neither of these is completely satisfactory when it is desired to compare heterogeneous seed lots. The disease index gives an indication of the maximum damage that might occur with seed planted in the field. Spore-load data constitute a more quantitative measure of contamination, but the washing-centrifuging method does not reach low spore loads. Glomerella damping-off has been produced by inoculation of seed with low spore loads. Experiments of the senior writer along this line will be reported elsewhere. Working with naturally contaminated seed, however, other factors may have to be taken into consideration, such as appressoria and dormant mycelia of Glomerella which would not be removed by washing seed, and other seed-borne organisms involved in seedling diseases.

The present study points toward a consideration of some practical importance. If seed cotton is not picked clean of trash in the eastern part of the cotton belt, the seed is likely to become contaminated with the anthracnose fungus, even though ginning removes most of the trash. This is particularly true when the trash is heavily infected. In the

ginning experiments, trash carrying a low spore load did not cause serious contamination of seed, although the amounts of trash added were about 6 times larger than those normally present in seed cotton. In 1941, average spore loads on seed and on trash were much higher than in 1940 (tables 9 and 10).

Carry-over of G. gossypii in the gin from one lot of seed cotton to the other has been investigated. This material is presented in the accompanying article, "The Dissemination of Fungus Spores from Contaminated Seed Cotton During Ginning in Relation to the Germination of the Seed and the Diseases of the Seedlings".

Summary

Samples of cotton seed and of trash were collected from gins in South Carolina during 3 successive seasons. Nearly all samples were found to be contaminated with the anthracnose fungus, Glomerella gossypii. Spore-load determinations revealed some effects of seasonal, sectional, and climatic factors on seed contamination.

Experiments have been conducted on contamination of seed by G. gossypii during ginning. Addition of infected trash to seed cotton prior to ginning produced contamination of seed. The degree of seed contamination depended on that of the trash. Carry-over of the fungus was obtained in lots ginned subsequent to heavily contaminated seed cotton.

It is concluded that contamination of seed in the gin accounts for much of the Glomerella damping-off of seedlings extant in the eastern part of the cotton belt, and that infected trash plays an important role in this contamination process.

THE DISSEMINATION OF FUNGUS SPORES FROM CONTAMINATED SEED COTTON DURING GINNING IN RELATION TO THE GERMINATION OF THE SEED AND THE DISEASES OF THE SEEDLINGS

Paul R. Miller

The purpose of the work herein reported was to determine the amount of Glomerella fungus contamination that results from ginning Glomerella-free cotton following contaminated cotton, and at the same time to secure information on spore loads of the common fungi occurring on the seed as related to germination of the seed and diseases of the seedlings.

For this seed spore load survey, varieties of cotton, grown in 4 locations, 2 of which -- Florence, South Carolina and Tifton, Georgia -- were known to be in anthracnose areas, and 2 of which -- Tipton, Oklahoma and Lubbock, Texas -- were in western areas where the disease usually does not occur, were sent to Stoneville, Mississippi and ginned in the order of listing in Table 12. Before ginning, seed cotton samples of these 41 varieties were bagged and transmitted to us along with the ginned samples. Spore load determinations of Glomerella gossypii, Fusarium spp., Diplodia spp., and Alternaria spp. were made utilizing methods previously described (P.D.R. 24: 85-92, 1940).

Table 12. Number of spores per seed compared with the percentage germination of the seed and the amount of disease on the seedlings

Sample no.	Number of spores								Percent germination	Disease index
	Glomerella gossypii		Fusarium spp.		Diplodia spp.		Alternaria spp.			
	a	b	a	b	a	b	a	b		
Florence, South Carolina										
1	15,000	20,000	300	1,800	0	200	200	600	69	50.5
2	10,000	20,000	600	10,000	0	0	0	1,800	72	59.5
3	5,000	10,000	1,400	10,000	0	400	0	1,200	58	46.5
4	5,000	5,000	1,800	5,000	0	0	0	400	77	51.5
5	10,000	5,000	1,000	10,000	0	0	0	1,000	79	51.5
6	25,000	35,000	600	10,000	0	0	0	800	87	73.2
7	25,000	12,000	1,000	10,000	0	400	0	1,200	75	66.5
8	25,000	15,000	5,000	8,000	0	0	0	800	74	58.0
Average	8,125	15,250	1,462	8,100	0	125	25	975	74	57.2
Tipton, Oklahoma										
9	800	0	200	5,000	0	0	0	5,200	69	5.2
10	200	0	1,200	800	0	0	0	1,600	84	9.5
11	0	0	600	5,000	0	0	200	3,400	81	2.7
12	200	0	1,200	800	0	0	200	6,000	89	7.5
13	0	0	5,000	20,000	0	0	200	4,400	79	5.5
14	0	0	10,000	15,000	0	0	400	6,800	78	1.5
15	0	0	5,000	20,000	0	0	200	4,000	84	4.0
16	0	0	1,200	25,000	0	0	400	2,400	93	11.0
17	0	0	600	10,000	0	0	0	2,600	88	2.2
18	0	0	800	15,000	0	0	400	2,600	76	23.2
19	0	0	25,000	160,000	0	0	0	6,800	70	4.2
20	0	0	5,000	10,000	0	0	200	3,200	94	4.2
21	0	0	15,000	25,000	0	0	0	2,200	84	2.7
22	0	0	5,000	20,000	0	0	0	2,800	84	1.7
23	0	0	600	15,000	0	0	0	4,400	88	1.2
24	0	0	800	5,000	0	0	400	2,600	74	2.5
Average	75	0	4,825	21,975	0	0	163	3,813	82	5.6

a Seed from ginned cotton.
b Seed from unginned cotton (seed cotton).

Table 12. Number of spores per seed compared with the percentage germination of the seed and the amount of disease on the seedlings (continued)

Sample no.	Number of spores								Percent germination	Disease index
	Glomerella gossypii		Fusarium spp.		Diplodia spp.		Alternaria spp.			
	a	b	a	b	a	b	a	b		
Tifton, Georgia										
25	10,000	20,000	5,000	15,000	0	0	0	3,800	69	58.0
26	5,000	10,000	1,400	25,000	0	200	400	400	79	58.5
27	800	5,000	600	30,000	0	0	0	1,000	80	57.5
28	800	15,000	1,000	35,000	0	200	0	600	77	59.0
29	5,000	5,000	800	20,000	0	0	0	800	79	59.7
30	15,000	5,000	600	10,000	0	0	200	800	85	59.7
31	800	600	5,000	15,000	0	200	0	2,000	80	57.2
32	400	400	5,000	20,000	0	0	0	1,200	92	53.2
33	400,000	80,000	600	15,000	0	800	0	1,200	70	68.7
34	5,000	800	800	5,000	0	200	0	800	86	55.2
Average	44,280	14,180	2,080	19,000	0	160	60	1,260	80	58.7
Lubbock, Texas										
35	400	0	800	1,000	0	0	0	1,600	77	8.5
36	200	0	200	1,000	0	0	200	600	81	13.5
37	0	0	0	600	0	0	0	200	86	10.7
38	0	0	0	25,000	0	0	0	1,200	82	7.5
39	0	0	15,000	20,000	0	0	0	1,800	74	6.2
40	0	0	1,000	5,000	0	0	200	1,800	72	3.7
41	0	0	0	200	0	0	0	600	65	3.2
Average	86	0	2,425	7,542	0	0	57	1,114	77	7.6

a Seed from ginned cotton.
b Seed from unginned cotton (seed cotton).

The germination percentage determinations of the various seed lots were made by D. M. Simpson. He used the standard laboratory germinating technique approved by the Association of Official Seed Analysts.

The disease indices were determined by Richard Weindling. Two replicates of 100 seedlings each for each ginned sample were grown in sterilized sand, and the disease index was derived by adding the number of dead plants to 1/2 the number of lesioned plants.

The results presented in Table 12 show that Glomerella gossypii occurred on the seed of all samples of both unginned and ginned cotton from Florence, South Carolina and Tifton, Georgia, but it was obtained from none of the

samples of the unginned cotton from Tipton, Oklahoma and Lubbock, Texas, and from only the first 4 of the ginned samples from Tipton and the first 2 from Lubbock. This shows that after ginning the infested cotton, spores left on the ginning equipment caused sufficient contamination of the originally disease-free seed to be detected by the spore-load determination method. It should be pointed out here that the anthracnose fungus occurred on a limited number of seedlings resulting from the seed of all ginned samples from Texas and Oklahoma, even though the mechanical method had revealed its occurrence on only 5 of the 23 samples. This inconsistency is attributed to the shortcomings of the technique and insufficient knowledge of the disease. However, the dependability of the method for determining spore loads in excess of 200 spores per seed has been established statistically. Perhaps it would not be reasonable to expect that all spores would be dislodged in the time that is practicable to devote to washing a sample, and it is not known how many spores per seed are necessary to cause seedling infection under varying environmental conditions.

It can now be said with considerable certainty that spores of Glomerella gossypii are disseminated from contaminated cotton during ginning and that this is an important factor in the widespread occurrence of this organism as a seedling parasite.

Fairly high spore loads of Fusarium spp. were obtained on all samples from all locations with the exception of 3 ginned samples from Lubbock, Texas, on which none were found. Spores of Diplodia spp. were not found on any of the ginned samples, but small spore loads were obtained on a few seed-cotton (unginned) samples from Florence, South Carolina and Tifton, Georgia. Alternaria spores were found on all unginned samples from the 4 locations and on about 1/2 of the ginned samples. Generally the number of spores per seed was lower on the ginned cotton than on the unginned. Table 12 shows that the average number of spores per seed for the 4 groups of organisms under consideration was considerably higher on the unginned (seed cotton) than on the ginned, although the number of bolls represented in an unginned sample was much lower than in a ginned one. This indicates that most of the spores on seed cotton are removed with the lint during ginning.

The recorded laboratory germination percentages show that generally there were only slight differences in the viability of the seed originating at the 4 locations. The differences, however, in the amount of disease that developed on the seedlings, depending upon the origin of the seed -- Eastern area versus Western -- were significant, and it seems that the differential Glomerella spore loads were largely responsible for these variations. The results of spore load studies conducted during the past 3 years have shown no relation between the size of Glomerella spore loads on a given sample of seed and the percentage germination of the seed. However, the spore load size does seem to influence the amount of post emergence damping-off that results when the seed are planted.

THE PROBABLE EFFECT OF HUMIDITY ON THE SURVIVAL AND SPORULATION OF THE ANTHRACNOSE FUNGUS ON COTTON

Paul R. Miller

The purpose of this study was to obtain information on the possible effect of climatic conditions on the ability of the anthracnose fungus (Glomerella gossypii) to survive during the summer and to sporulate. The results tend to show that humidity is an important factor (Table 13 and Figure 2).

Glomerella gossypii-contaminated seed from a common lot of Stoneville 5 variety of cotton was planted at 20 locations indicated in Figure 2. When harvested, this cotton was sent to one place for ginning. Utilizing the technique previously described (P.D.R. 24: 85-92, 1940) anthracnose fungus spore load determinations were made. Results given in Figure 2 show that relatively high spore loads were secured from seed produced at locations in the more humid belt, i.e., in areas near the coast. Generally, low spore loads were obtained from locations in the inland sections of the Eastern cotton belt where somewhat lower humidity prevails. No spores were found on cotton originating in the sub-humid and semi-arid belts of Texas and Oklahoma. Very similar results were obtained when a like study was conducted with seed of the same variety planted in 1939 at most of the same locations.

The distribution of anthracnose indicated in Figure 2 corresponds to that determined during the cotton seedling and boll disease surveys.

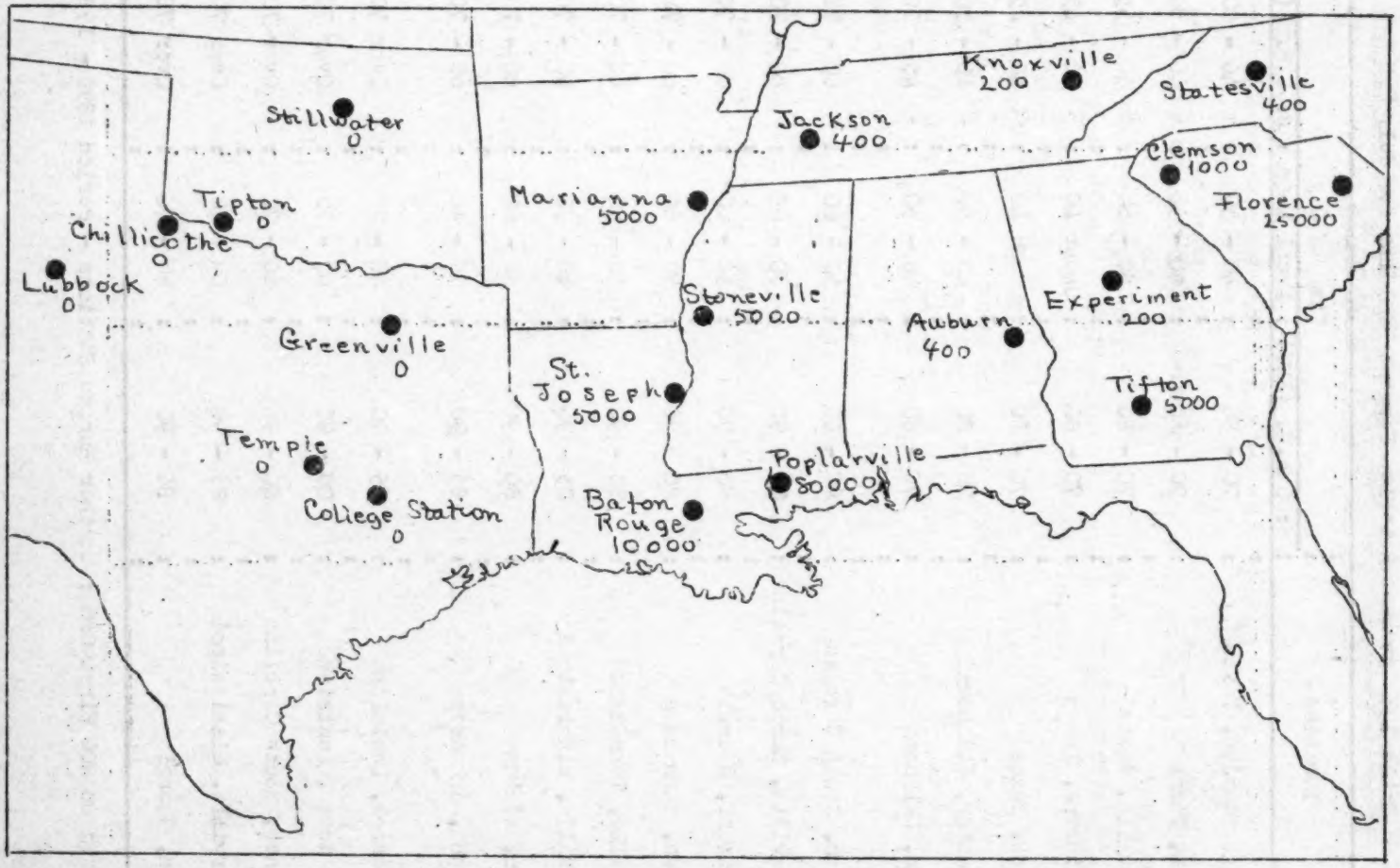

Figure 2. Number of anthracnose (Glomerella gossypii) spores per seed on cotton grown in 1941 from the same seed lot (Stoneville 5) at 20 locations

Table 13. Average Relative Percent Humidity for July [a]

Location	Time of Day		
	8:00 A.M. (EST)	Local Noon	8:00 P.M.(EST)
College Station, Texas	70 - 80	40 - 50	40 - 50
Temple, Texas	70 - 80	40 - 50	40 - 50
Greenville, Texas	70 - 80	50 - 60	50 - 55
Chillicothe, Texas	70 - 80	Under 40	40 - 45
Lubbock, Texas	70 - 80	Under 40	40 - 45
Stillwater, Oklahoma	70 - 80	40 - 50	40 - 50
Tipton, Oklahoma	70 - 80	40 - 50	45 - 50
Clemson, South Carolina	80 - 90	50 - 60	60 - 70
Statesville, North Carolina	80 - 90	50 - 60	60 - 70
Experiment, Georgia	80 - 90	50 - 60	60 - 70
Jackson, Tennessee	80 - 90	50 - 60	60 - 70
Knoxville, Tennessee	80 - 90	50 - 60	60 - 70
Stoneville, Mississippi	85 - 90	60 - 70	60 - 70
Auburn, Alabama	80 - 90	50 - 60	60 - 70
Marianna, Arkansas	85 - 90	50 - 60	60 - 70
St. Joseph, Louisiana	85 - 90	60 - 70	Over 70
Baton Rouge, Louisiana	80 - 90	60 - 70	Over 70
Florence, South Carolina	80 - 90	60 - 70	Over 70
Poplarville, Mississippi	85 - 90	Over 70	Over 70
Tifton, Georgia	80 - 90	60 - 70	Over 70

[a] Based on 200 First Order Weather Bureau Stations - Period 1899 - 1938.